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Procedia - Social and Behavioral Sciences 132 (2014) 87 – 91

**Procedia**  
Social and Behavioral Sciences

6<sup>th</sup> International Conference on Intercultural Education “Education and Health: From a transcultural perspective”

## Hormonal changes analysis by effects of Horses Assisted Therapy in the autistic population

Carmen Tabares Sánchez<sup>a</sup>, Florencio Vicente Castro<sup>a</sup>, Susana Sánchez Herrera<sup>a</sup>, Javier Cubero Juárez<sup>b</sup>

<sup>a</sup> University of Extremadura. Department of Psychology and Anthropology Avd. Elvas s/n, Badajoz 06006, Spain

<sup>b</sup> University of Extremadura. Laboratory of Health Education, Experimental Sciences Education Area, Avd. Elvas s/n, Badajoz 06006, Spain

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### Abstract

The aim of this work is to objectively verify whether Horses Assisted Therapy leads to an improvement of social attitudes in people with autism, analysing, through saliva samples, by the enzyme immunoassay technique ELISA, the hormonal levels of Cortisol and Progesterone and infer the behaviour of Oxytocin, before and after the therapy sessions. A non-random convenient sample is performed, approaching a group of eight autistic children (n=8).

The results show a statistically significant decrease in Cortisol levels in saliva in Pre-Therapy in relation to Post-Therapy (Pre-Therapy  $8.71 \pm 1 \text{ ng/ml}$  vs Post-Therapy  $6.58 \pm 0.63 \text{ ng/ml}$ ). Additionally, the Progesterone levels in Pre-Therapy are significantly less than in Post-Therapy (Pre-Therapy  $32.78 \pm 7.60 \text{ pg/ml}$  vs Post-Therapy  $60.05 \pm 6.72 \text{ pg/ml}$ ). This leads us to determine in an approximate way the behaviour of the Oxytocin hormone, whose levels in Pre-Therapy will be less than in Post-Therapy.

We therefore conclude that Horses Assisted Therapies lead to an improvement of social attitudes in the treatment of people with autism.

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Selection and peer-review under responsibility of HUM-665 Research Group “Research and Evaluation in Intercultural Education”.

**Keywords:** Horse Assisted Therapy, Autism, Oxytocin, Cortisol and Progesterone

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### 1. Introduction

Horses Assisted Therapy is a therapy which uses equines in the treatment of disorders associated with several neurological and neuromuscular pathologies.

It can be defined as “methodology of complimentary rehabilitation designed, executed and evaluated by a specialist technician in which the horse and its entire environment are used to intervene on different areas which make up the complete development of the person” (Vives, 2010).

Due to the horse's unconditional devotion, the experience becomes a great motivational factor for the patient. Therefore, children with disorders such as autism, which affects the normal development of the brain, manifesting itself in social, communication and imagination abilities problems, will benefit from this intervention.

Essays that objectively verify whether Horses Assisted Therapy, so spread in the last few years, is really optimal on a physical, psychological and social level for the treatment of autism are scarce, both nationally and internationally. We consider that verifying in an objective way the benefits of these therapies could be a great scientific contribution for the development of this disorder and for the Horses Assisted Therapy as such.

We therefore propose to carry out an analysis of hormonal variations on subjects with autism and study the behaviour of such hormones before and after Horses Assisted Therapy sessions.

Regarding the hormonal analysis, the Oxytocin is related to sexual patterns and motherly and fatherly conducts, but is also associated to affectivity, tenderness and the act of touching. In the brain, it appears to be involved in recognising and establishing social relationships and is related to the creation of trustworthy relationships and generosity (Bales, Boone, Epperson, Hoffman & Carter, 2011; Kosfeld, Heinrichs, Zak, Fischbacher & Fehr, 2005).

In relationship to autism, we find authors such as Modahl et al. (1998) or Green et al. (2001), who declare that there are significantly less levels of Oxytocin in blood plasma in autistic subjects and a decrease in repetitive conducts spectrum when the hormone is administered. Apparently, and especially in severe cases, these subjects present low hormonal levels of Oxytocin. Equally, it was shown that Oxytocin helped to retain the ability of evaluating the emotional meaning of speech intonation.

To measure the levels of Oxytocin in blood plasma is complicated, as it can be released in an irregular manner and therefore it varies. However, different studies have found abnormalities in the levels of plasma and the subgroup of children with autism, who are the most socially isolated, tend to have the lowest levels of Oxytocin (Hollander et al., 2006).

This hormone seems to be also involved in recognising and establishing social relationships and could be connected to the forming of reliable relationships and generosity among people. This is confirmed by authors such as Kirsch et al. (2005)

Finally, following Engelmann et al. (2000), Oxytocin would be part of a neuroendocrine system with activity in the beneficial effects of positive social stimuli and this could be decisive in autism, where the social area is, among others, one of the most affected.

On the other hand we find other hormones, such as cortisol and progesterone, both closely related to Oxytocin as regards its behaviour inside the organism (Amico, Seitchik & Robinson, 1984).

As regards to Cortisol, a clear interrelation between Oxytocin and this hormone has been found (Kovacs, Sarnyai y Szabo, 1998; Uvnas-Morberg, Bjokstrand, Hillegaart & Ahlenius, 1999; Agren y Lundeberg, 2002; Ditzen et al, 2009) since Oxytocin counteracts the effects of Cortisol, the stress hormone (Legros, 2002).

In other studies, however, such as Stephanie Brown's (2009), Progesterone has been established as a possible part of the neuroendocrine basis for social attachment, having the same behavioural tendency as Oxytocin.

The strategy, therefore, to infer the behaviour of Oxytocin, is to use the above (Cortisol and Progesterone) to quantify through laboratory clinical analysis the observation of hormonal behaviour.

For all these reasons, the aim of the study has been to verify whether the Horses Assisted Therapy indeed leads to an improvement of social attitudes in people with autism, through the analysis of saliva hormonal levels: Cortisol and Progesterone, which are closely linked to Oxytocin levels.

## 2. Materials and methods

**Subjects.** A non-random convenient sample is performed, approaching a group of eight autistic children (n=8); they are all male, therefore eliminating any contamination deriving from sex, mainly dealing with hormonal behavior. The children ages range from 5 to 15 years old, and we reached them through the Association APNABA in Badajoz, who attend Horse Assisted Therapy sessions under the direction of specialists in this technique from

the Association of Zootherapy of Extremadura.

**Salivary samples.** Salivary samples were collected in Salivette collectors, distributed in Spain by Sarstedt (La Roca del Valles, Barcelona). The material was transferred to the laboratory in special containers, refrigerated, provided with cold chain interruption control, meeting the UN 3373 standard. Once in the laboratory, they were frozen and stored in special freezers at  $-72^{\circ}\text{C}$  until the day of analysis.

**Analytical methods.** To determine the hormone levels, a competitive enzyme immune essay method (ELISA) was used, through electrochemoluminescence in a TECAN Genesis RPM 150 modular reader, with wavelength a 450 nm absorbance. The equipment, both for Cortisol and progesterone, are from Demeditec Diagnostics (Germany), distributed in Spain by Materlab (Madrid). All the analytical determinations were performed in duplicate.

**Experimental design.** The material collection has been two-way: before and after the Horse Assisted Therapy sessions. The time difference between Pre-therapy and Post-therapy is an hour, each session carried out by each individual. The saliva was always collected the same day of the week and at the same time for each individual, to avoid a possible hormonal variation due to circadian rhythms typical in hormones.

Having based our method on the differences of the Pre-Therapy and Post-Therapy hormone levels, we cancel any possibility of strange variable influence through food ingestion or mouth wash, among others, which would indeed have great influence if they were isolated determinations. Additionally, we avoid any interference due to each individual's basal levels –normal or not.

**Statistical Analysis.** The therapy session was conducted over 12 weeks. The statistical data used to study in the analysis were the Media and the Standard Error ( $X \pm \text{SE}$ ). For the Inferential analysis we studied, because of the nature of the data, paired comparisons through the Student's t-test, with a statistical meaning level:  $p \leq 0.05$ .

### 3. Results

The concentrations have been shown in pg/ml in the case of the Progesterone hormone and ng/ml in the case of the Cortisol hormone.

Table 1. General levels ( $X \pm \text{SE}$ ) of Cortisol (ng / ml) and Progesterone (pg / ml) in saliva in individuals with autism Pre and Post Horses Assisted Therapy in 12 weeks treatment (n = 8).

GENERAL RESULTS			
CORTISOL (ng/ml)		PROGESTERONE (pg/ml)	
Pre-Therapy	Post-Therapy	Pre-Therapy	Post-Therapy
8.71 $\pm$ 1	6.58 $\pm$ 0.63	32.78 $\pm$ 7.6	60.05 $\pm$ 6.72

As we observed in the previous table, the main results indicated that equine therapy decreased the levels of salivary Cortisol (Pre-Therapy 8.71 $\pm$ 1ng/ml vs Post-Therapy 6.58 $\pm$ 0.63ng/ml). And also increased the levels of salivary Progesterone (Pre-Therapy 32.78 $\pm$ 7.60pg/ml vs Post-Therapy 60.05 $\pm$ 6.72pg /ml).

### 4. Limitations in the study

The first limitation we face was the size of the study population. Needing a particular sample, in our case people diagnosed with ASD and also that this population receives as concrete as Horses Assisted Therapy, has conditioned us to have the only available population. Therefore has been a non-random convenient sample is performed, approaching a group of eight autistic children.

This limitation has conditioned us another, and that is that we have been forced to stick exclusively to boys, they are all male. Therefore eliminating any contamination deriving from sex, mainly dealing with hormonal behaviour.

Finally, due to the nature of our study, we do not believe it appropriate to analyze a group of control, it is not necessary because we rely on individual comparisons of each subject on two occasions, Pre-Therapy and Post-Therapy

## 5. Discussion & Conclusions

Once confirmed the tendency of the data, it can be observed that Horses Assisted Therapy reduces the stress levels in the organism, which manifests itself in a decrease of the levels of Cortisol hormone after therapy. The tendency of the Progesterone, on the other hand, is to increase the levels in the body after therapy sessions, which translates into an improvement in emotional canals. With all these results, and given the hormonal correlation we start from, we can infer that Oxytocin levels in the organisms of these subjects increase as a result of this intervention. Therefore we determine that emotional canals created through contact with the horse in a group of children with autism are effective.

Also, we think in a possible effect "Time Dependent" of this therapy. And we can conclude that the Horse Assisted Therapy for the population with ASD generated leads to an improvement in social attitudes, and it is confirmed with the effective modulation of the implicating emotional hormones.

This means that Horses Assisted Therapy is a form of treatment which, as any other form of therapy, needs a more or less defined method to reach goals. Additionally, in order to achieve those goals in the therapeutic treatment of any given disorder, such as in our case the treatment of ASD, therapy must linger on in time. It is not about isolated sessions, instead it must be carried out in a continuous and constant way, and performed by qualified professionals, as otherwise we could not call this technique a "therapy".

## Acknowledgements

- Diputación Provincial of Badajoz, Spain.
- APNABA Association, Badajoz, Spain.
- Association of Zootherapy of Extremadura, Spain.

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